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Dermatological Application of PAMAM – Vitamin Bioconjugates and Host-Guest Complexes – Vitamin C Case Study

Stanisław Wołowiec[1], Marek Laskowski[1],
Barbara Laskowska[1], Agnieszka Magoń[2],
Bogdan Mysliwiec[2] and Marek Pyda[2]
[1]*Department of Cosmetology,*
University of Information Technology and Management,
[2]*Faculty of Chemistry, Rzeszów University of Technology,*
Rzeszów
Poland

1. Introduction

Stoichiometry of reaction describes the quantitative relationships among substances as they participate in chemical reactions. Furthermore the term stoichiometry is used to describe the quantitative relationship among elements in compounds. This is also valid in the quantitative description of so-called weak complexes formed between molecules through non-covalent and non-ionic interactions. They generally arise from hydrogen bonding or Van der Waals interactions and are very common in biological chemistry, like in the case of enzyme-substrate, enzyme-inhibitor, or enzyme-coenzyme complexes. In less specific systems, stoichiometry may be referred to the number of small molecules interacting with one macromolecule to form complex structure. Depending on the interaction type between the components of the complex they are often desribed as host (macromolecule, H) – guest (small molecule, g) complexes. The host-guest complexes can sometimes be isolated as compounds of defined strochiometry, however their dissolution always leads to establishing an equilibrium between complexes and free guest, according to the scheme:

$$Hg_n \Leftrightarrow Hg_{n-1} + g \Leftrightarrow Hg_{n-2} + 2g \Leftrightarrow Hg_{n-3} + 3g \ldots\ldots \Leftrightarrow H + ng \quad (1)$$

described by overall formation constant:

$$K = [Hg_n]/[H][g]^n \quad (2)$$

This weak interaction between H and g leads to the formation of multiple complexes Hg_n, with n = 1, 2, 3, …. n, depending on g concentration, which is in excess compared to H one. Its larger amount is necessary to determine n_{max}, i.e. the maximum amount of guest

molecules which are able to interact with one host macromolecule. Thus the n_{max} is often referred to as host-guest complex stoichiometry or host capacity in relation to specific guest.

Commonly employed molecular complex is the one used in iodometric titration, where the equivalent point is determined by the deeply blue color of starch (macromolecular host) and iodine (guest) complex disappearance with the iodine completely consumed by thiosulfate (Rundle & Frank, 1943).

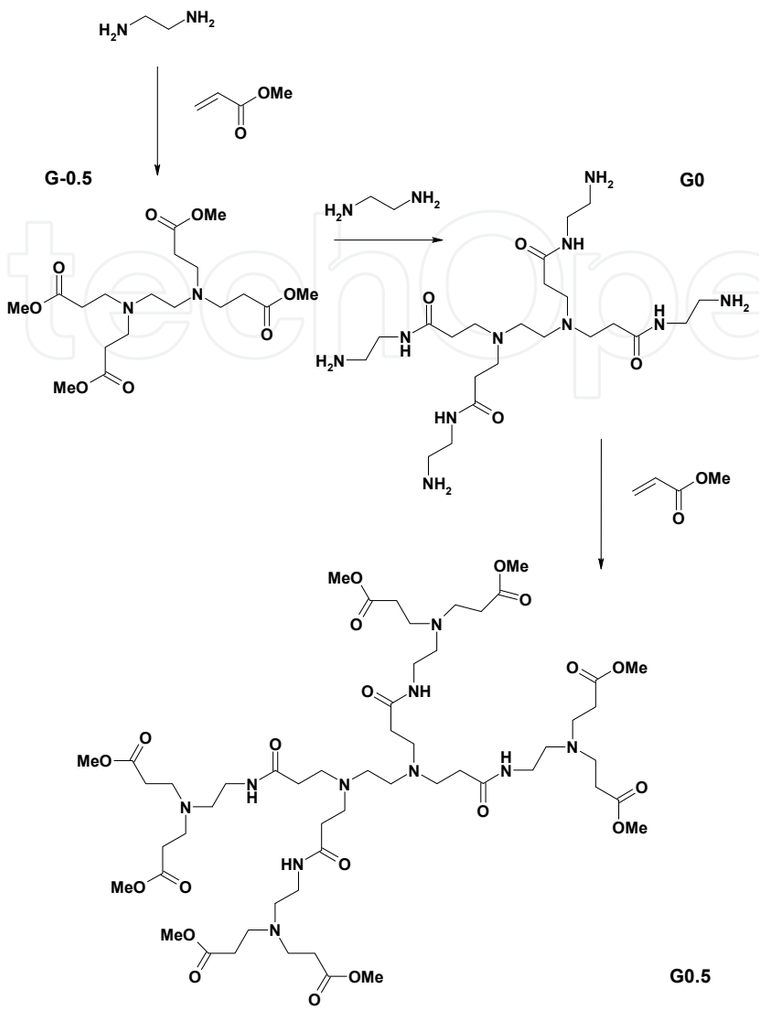
Starch is a glucose polymer containing mainly α(1-4) glycosidic bonds with an amylose moiety forming polyiodide complexes with maximum absorbance at 620 nm. Being starch a polydisperse polymer, the equilibrium constant for the blue iodine complex formation is actually the averaged value (Saenger, 1984).

More adequate formation constants can be determined for monodisperse macromolecules, such as dendritic polymers. The dendrimer chemistry developed very fast over the last two decades, and it has been the subject of many reviews due to dendrimers numerous applications, such as: nanomaterials for molecular electronics, photonics, sensors (Astruc et al., 2010, Lo & Burn, 2007) and nanomedicine (D'Emanuele & Attwood, 2005, Caruthers et al., 2007). Polyethyleneimine (PEI), polyester (PES) and polyamidoamine (PAMAM) dendrimers invented in Frechet's and Tomalia's group macromolecules own a signifficant position among dendrimeric molecules (Frechet et al., 1995, Tomalia , 2005a and b).

2. Polyamidoamine dendrimers (PAMAM)

2.1 General characteristics of PAMAM dendrimers

The dendritic molecules are globular shaped monodisperse objects. The polyamidoamine dendrimers (PAMAM) were synthesized in Tomalia's group (Tomalia et al., 2003, Tomalia, 2005). The PAMAM dendrimers of full generation posses several surface amine groups which make them perfectly soluble in water. They can be obtained by alternate addition of methyl acrylate to amine (in our study ethylenediamine has been used as starting diamine core) resulting in doubling the surface methyl propionate groups, followed by condensation of ester groups with diamine (ethylenediamine being the best choice). The latter step introduces further amine function on surface. Thus the full generation (Gn) PAMAM dendrimers posses: 8, 16, 32, and 64 surface amine groups in G1, G2, G3, and G4, respectively. The synthetic pattern is shown in Scheme 1. The hydrodynamic diameters of full generation PAMAM dendrimers are: 1.5, 2.2, 2.9, 3.6, and 4.5 nm for G0, G1, G2, G3, and G4, respectively. The surface structure of full generation dendrimers can be widely modified by surface amine groups conversion, resulting in change in their solubility and permeability properties (Jevprasesphant et al., 2003, Imae et al., 2000). The PAMAM dendrimers are molecules with inner cavities, which are able to absorb small molecules to form host-guest complexes. During the synthesis of PAMAM dendrimers in methanol, the final removal of this solvent from the dendrimer spherical macromolecule requires prolonged vacuum evaporation. PAMAM dendrimers ability to encapsulate hydrophobic guests was extensively used as a strategy for promoting water insoluble drugs transportation into tissues and cells (vide infra).



Scheme 1. The scheme of synthesis of PAMAM dendrimers on ethylenediamine (en) core. Addition of methyl acrylate to en results in formation of -0.5 half-generation PAMAM dendrimer (G-0.5) with four methyl ester groups on surface. The condensation of G-0.5 with four en equivalents leads to full-generation PAMAM dendrimer (G0). Further addition of methyl acrylate gives G0.5 PAMAM dendrimer, which upon next condensation with eight en equivalents results in obtaining full generation PAMAM dendrimer G1 (not shown). Repeated sequence of addition/condensation protocol leads to G1.5, G2, G2,5, G3 and so on.

2.2 Medical applications of PAMAM dendrimers

PAMAM dendrimers are relatively low toxic (Jain et al., 2010, Mukherjee et al., 2010, Kolhatkar et al., 2007, Hans & Lowman, 2002). Therefore they are extensively studied as drug carriers. There are two strategies commonly applied: 1) diffusion of prodrug covalently bound to the dendrimer, and 2) diffusion of drug encapsulated in the dendrimer. Thus, the PAMAM dendrimers act as solubilizers for anti-inflamatory drugs *ketoprofen* (Yiyun et al., 2005), *ibuprofen* (Milhem et al., 2000) and *indomethacin* (Chauhan et al., 2003) and promote

their prolonged release both *in vitro* and *in vivo* (Na et al., 2006, Kolhe et al., 2003). Similar concept was used to increase solubility and uptake flux of several anticancer drugs, like *methoxyestradiol* (Wang et al., 2011), *adriamycin* and *methotrexate* (Kojima et al., 2000), a plant alkaloid *camptothecin* (Cheng et al., 2008), *doxorubicidin* (Papagiannaros et al., 2005), and *dimethoxycurcumin* (Markatou et al. 2007). Moreover, *nifedipine* - a calcium channel blocking agent (Devarakonda et al., 2004, 2005), a loop diuretic *furosemide* (Devarakonda et al., 2007, antipsychotic drug *risperidone* (Prieto et al., 2011), a hypolipidemic drug (Kulhari et al., 2011), antibiotic *quinolones* (Cheng et al., 2007), antibacterial *sulfamethoxazole* (Ma et al., 2007), as well as ophtalmic drugs *pilocarpine nitrate* and *tropicamide* (Vandamme & Brobeck, 2005) were also supported by PAMAM dendrimers as carrier. Surprisingly PAMAM – drug complexes were proven to be effectively transdermal increasing the flux of *5-fluorouracil* - a drug for treatment of psoriasis, premalignant and malignant skin conditions (Venuganti & Perumal, 2008, Bhadra et al., 2003) of dermatologically important vitamins, like *nicotinic acid* Cheng & Xu, 2005) and *riboflavin* (Filipowicz & Wołowiec, 2011) as well as of *psoralene* – the photosensitizer used for treatment of psoriasis (Borowska et al., 2010).

A more advanced strategy is based on a covalent bonding of the prodrug, bioconjugates being extremely promising for site-directed therapy. Thus, the PAMAM bioconjugates with drugs synthesized and tested *in vitro* so far are the one with *methotrexate* and *folate* (Patri et al., 2005, Chandrasekar et al., 2009), *ibuprofen* (Kolhe et al., 2006, Kurtoglu et al., 2010), *methylprednisolone* (Khandare et al., 2005), *adriamycin* (Kono et al., 2008), *triamcinolone* (Ma et al., 2009), *propranolol* (D'Emanuele et al., 2004), *5-aminosalicylic acid* (Wiwattanapatapee et al., 2003), and especially important for skin treatment *phosphorylcholine* (Jia et al., 2011), *cholic acid* (Zhang et al., 2011), *biotin* (Yang et al., 2009), and *riboflavin* (Thomas et al., 2010). Similar studies are currently being performed in our laboratory to clarify the skin penetration of vitamins supported by PAMAM dendrimers. In some cases PAMAM dendrimers act as solubilizers and enhance the skin load, while in other cases the vitamin flux decreases. Here we present the latest results on vitamin C permeation in the presence of PAMAM dendrimers.

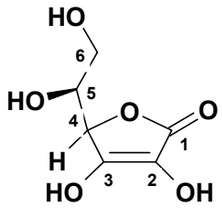
3. PAMAM dendrimers as carriers for vitamin C

Ascorbic acid (vitamin C) is an essential nutrient protecting living tissues from oxidation processes by free radicals and reactive oxygen derived species. It is also an important co-factor in collagen synthesis, hence present in most cosmetic products. The bioavailability of topically applied vitamin C is very high compared with other dermatologically important vitamins and depends on the specific delivery system, like o/w, w/o emulsions and hydrogels (Rozman et al., 2009) and liposomes, niosomes or solid lipid nanoparticles as dispergents (Kaur et al., 2007) as well as microemulsions (Kogan and Garbi, 2006). Due to its instability vitamin C is usually present in cosmetics as ester derivatives which are thermally and oxidatively more stable. Besides they also promote diffusion through epidermis. Though ascorbic palmitate is generally used, recently some other esters (Shibayama et al., 2008, Kumano et al., 1998, Tai et al., 2004) and even tandem retinyl ascorbate (Abdulmayed and Herda, 2004) were synthesized and their skin bioavailability tested.

The permeation rate through whole mouse skin was estimated as 3.43 (± 0.74) $\mu g/cm^2/h$ (Lee and Tojo, 1998) assuming that the least permeable barrier of *stratum corneum* was a

matrix of intercellular lipid due to its low water content. This also demonstrated that the rapid permeation of vitamin C when released from delivering formulation is not necessarily a cosmetic target. Macromolecular carriers can be used for controlling the transdermal diffusion rate of cosmetic ingredients; polyamidoamine (PAMAM) dendrimers, widely used as transdermal carriers for drugs have been shown to be amongst the best candidates (Svenson, 2009). For water-insoluble vitamins, like A and E, they can serve as solubilizers, while for water soluble vitamins, like ascorbic acid (**1**), they might allow to increase the skin load. In such a case the PAMAM dendrimers could be used as valuable additives for cosmetic creams.

Here we present the results on simple host-guest chemistry between PAMAM dendrimers and **1** in solution and in neat dendrimers **G2,5**, **G3**, **G3,5**, and **G4** studied by the ^{1}H NMR and differential scanning calorimetry (DSC) methods in order to establish the proper stochiometry of host-guest complexes available for cream formulation. The preliminary *in vitro* studies on diffusion of **1** absorbed in PAMAM dendrimers through artificial model membrane (polyvinydifluoride, PVDF) and pig ear skin (PES) were also performed.



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3.1 Materials and methods

3.1.1 Reagents

Ascorbic acid (vitamin C, **1**, MW = 176.12 g/mol, m.p. = 190 - 194°C with decomposition) was used as received. All solvents and reagents were of reagent grade purity (Aldrich) and used without further purification.

3.1.2 Syntheses of PAMAM dendrimers

PAMAM dendrimers of generation 2, 2,5, 3, 3,5, and 4 (**G2**, **G2,5**, **G3**, **G3,5**, and **G4**, respectively) on ethylenediamine core were synthesized according to the published method by alternate addition of methyl acrylate to **Gn** and condensation of ethylenediamine with **Gn,5** [Tomalia et al., 2003]. The dendrimers were characterized by the ^{1}H and ^{13}C NMR spectra in deuterium oxide and in methanol-d_4 using standard 1-D and 2-D COSY, HMBC, and HSQC methods with 500 MHz Bruker UltraShield spectrometer to confirm their purity.

3.1.3 Solubilization of 1 in methanol containing host dendrimers

The solubility of **1** in methanol-d_4, estimated by reference chloroform addition into the saturated solution is 0.36 mol dm^{-3}. ^{1}H and ^{13}C resonances assignement has been performed based on 1-D and 2-D NMR experiments.

^{1}H NMR (chemical shift [ppm], intensity, multiplicity, assignment): 4.88 ([1H], d, H^4); 3.90 ([1H], d of t, H^5); 3.68 ([2H], d, H^6); ^{13}C NMR: 172.1 (C^1); 153.4 (C^3); 118.5 (C^2); 75.5 (C^4); 69.2 (C^5); 62.2 (C^6).

The solubilization of **1** in presence of dendrimers was studied by straight addition of solid **1** into 700 µL of **G3**, **G4**, **G2,5** or **G3,5** solutions at variable concentration in methanol-d_4 after added a measured amount of chloroform as quantitative reference. The H^4 doublet of (**1**) shifted from 4.88 to 4.73 ppm upon dissolving in solution containing **G3** or **G4** dendrimers. Partial precipitation of the complex occurred when added amount of **1** added reached **1**:**G3** and **1**:**G4** ratio equal to 3:1. The analysis of separated solid indicated that the composition of water-soluble solid complex was 3:1, independently on dendrimers concentration. Solubilization of **1** in methanol-d_4 in presence of **G2,5** or **G3,5** showed no complexes precipitation within studied range of dendrimer concentration (up to 0.003 mol·dm^{-3}); the solubility of **1** depending linearly on it.

Linear regression of **1** versus **G2,5** and **G3,5** concentrations are shown in Figure 1 and 2, respectively. The proton resonances in the ^{1}H NMR spectrum of **1** in solutions containing **G2,5** or **G3,5** were not shifted compared with **1** in pure methanol-d_4.

3.1.4 Differential scanning calorimetric studies on PAMAM-1 stoichiometry

Glass transition temperature T_g was examined using heat-flux differential scanning calorimeter DSC, Q1000™ from TA Instruments, Inc., equipped with a mechanical refrigerator from temperatures 183.15 K (-90°C) to 393.15 K (120°C) (dry nitrogen gas with a flow rate of 50 cm^3·min^{-1} was purged through the DSC cell in the instrument and cooling was accomplished with a refrigerated cooling system). Samples of neat **G2,5**, **G3**, **G3,5**, and **G4** dendrimers were examined as well as host-guest complexes prepared by dissolving **Gn** or **Gn,5** in methanol followed by addition of measured amount of **1** and extensive vacuum evaporation. The oily samples of **1** and **Gn** or **Gn,5** exhibited a higher T_g value than the neat dendrimers (Table 1) within **1**:**PAMAM** ratio 6:1 for **G2,5** and 3:1 for other generations of PAMAM dendrimer. The original T_g of pure dendrimers was recovered once exceeded these molar ratios, with a subsequent lost of the sample homogeneity.

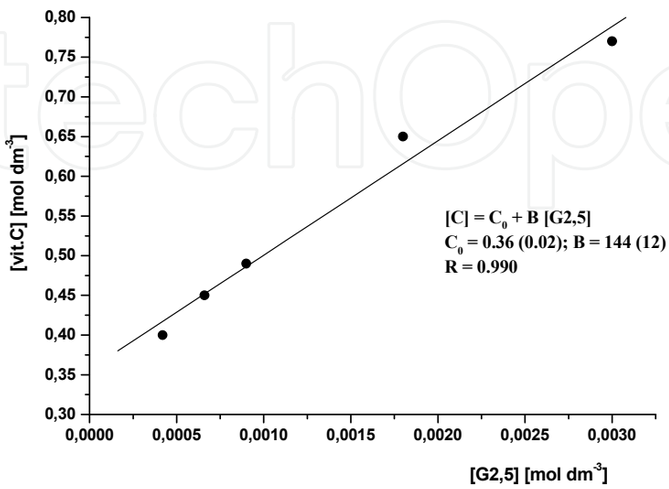

Fig. 1. The dependence of solubility of 1 in methanol containing variable concentration of G2.5.

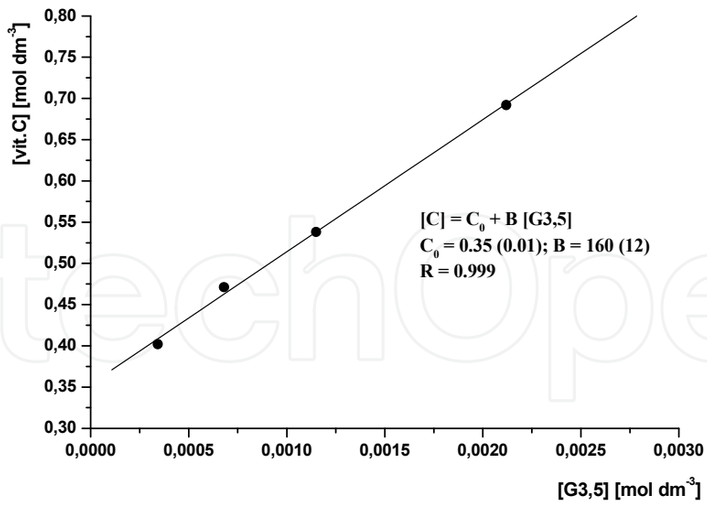

Fig. 2. The dependence of solubility of 1 in methanol containing variable concentration of G3.5.

1 : Gn ratio	G2,5	G3	G3,5	G4
0:1	229	253	224	248
1:1	243	265	240	263
2:1	243	268	251	269
3:1	247	267	255	259
4:1	246	257	224	249
5:1	244	254		248
6:1	250			
7:1	225			

Table 1. Glass transition temperatures [K] for Gn dendrimers and mixtures of Gn and 1.

3.1.5 *In vitro* permeation of Gn-1 and Gn,5-1 complexes

Permeation of **Gn-1** and **Gn,5-1** complexes was studied using Franz diffusion assembly (Thermo Scientific (UK) model DC 600 equipped with 6 cm^3 acceptor compartments). The o/w emulsion was used as donor. The emulsion was prepared using cetearyl alcohol (1.5 g), Brij 72 (1.2 g), Brij 58 (0.3 g) as emulsifiers, vaseline (5.0 g), stearine (0.5 g), glycerin (1.5 g) and water (40.0 g). The samples containing **Gn-1** complexes were prepared on 1 g scale by dissolving **1 (***ca* 5 mg) and **Gn** (*ca* 50 mg) in 1 g of the emulsion. Preliminary samples were obtained by dissolving the host-guest complexes **Gn-1** (prepared by mixing **1** and **Gn** in methanol followed by vacuum evaporation) in emulsion on the same scale. No difference in the permeation studies were noticed related to the protocols. For the analysis ca 250 mg samples were mounted over commercial polyvinydifluoride membrane (PVDF, 0.125 mm thickness) or prepared pig ear skin membrane (PES, 0.55 mm thickness), with 0.067 M phosphate buffer pH = 7.4 : ethanol 7:3 v/v. as receptor medium. The progress of diffusion was monitored spectrophotometrically at 264 nm using the extinction coefficient calculated for the solution of **1** in receptor solution ($1.1 \cdot 10^4$, Figure 3). The receiving solution was

stirred magnetically with 1000 rpm at 32°C. 10 ml aliquots of receptor solution were taken at 0.5 hour or longer time intervals and the receiver compartment was filled with 6 ml portion of a new receptor solution. The experiments were carried on until at least 10% of initial amount of **1** was received in receptor solution. The results were analyzed calculating the flux in [μmol·hour^{-1}·cm^{-2}]. The active area of membrane determined by size of the ring in Franz cell was 0.176 cm^2. The cumulated amount of **1** received as a function of diffusion time was crucial to determine the diffusion properties of **Gn-1** complexes. The time of 10% diffused **1** ($\tau_{0.1}$) was used as quantitative parameter to compare diffusion efficency. Permeation experiments were repeated 7 times. The mean standard deviation and workup of data were performed as previously (Filipowicz and Wołowiec, 2011).

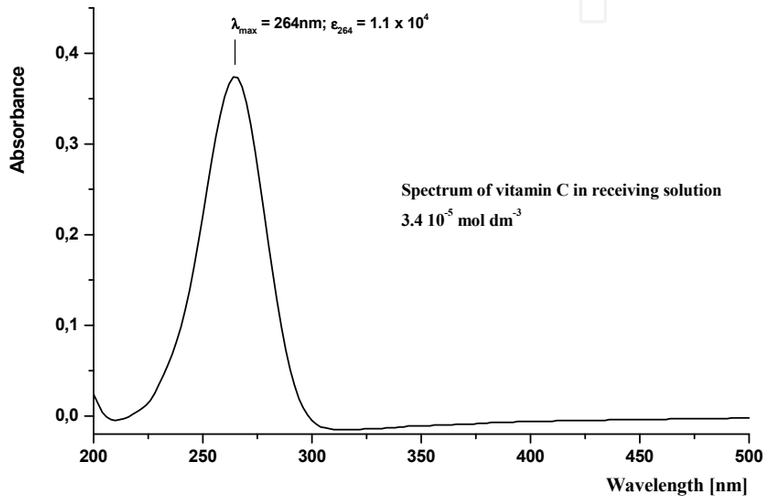

Fig. 3. The UV-Vis spectrum of **1** in receptor solution.

3.2 Results and discussion

3.2.1 Solubilization of 1 in presence of dendrimers studied by the ^{1}H NMR

Ascorbic acid (**1**) is well soluble in water, while its solubility in methanol-d_4, determined by ^{1}H NMR spectroscopy is 0.35 mol·dm^{-3}. It increases when bound to PAMAM dendrimers **G2**, **G3**, **G4**, **G2,5** and **G3,5**, respectively. When **1** is dissolved in methanol-d_4, the doublet resonance of H^4 shifts considerably from 4.80 into 4.62 ppm when **1**:**G4** ratio is 1:1. The species formed between **1** and **G3** or **G4** dendrimers become insoluble when the **1**:**Gn** ratio reaches 3:1 and 6:1 for **1**:**G3** and **1**:**G4**, respectively. The isolated complexes are readily water soluble. Presumably their stoichiometry does not correspond to the dendrimers ability to absorb small molecules of ascorbic acid, rather than to the limit of their solubility in methanol.

Opposite to **G3** or **G4** the **G2,5** and **G3,5** dendrimers which have similar sizes and molecular weights act as effective solubilizers of **1** in methanol. They are able to solubilize *ca* 144-160 molecules of **1** per molecule of dendrimer, almost independently on the host size. When methanol is evaporated from such a samples, the separation of **1** from dendrimer is observed resulting in formation of non-homogeneous mixture. In order to establish the stoichiometry of neat host-guest complexes another method was used.

3.2.2 Differential scanning calorimetric studies of neat complexes of 1 with dendrimers

Recently we have used DSC method to evaluate the stoichiometry of host-guest complexes between PAMAM dendrimers and *8-methoxypsoralene* (Borowska et al., 2010). Water insoluble host formed oily host-guest complexes which revealed higher temperature of glass transition (T_g) than PAMAM dendrimers. When the guest amount was increased above the host maximum capacity, the separation of PAMAM dendrimers and guest took place, followed by T_g returning to its original value. Similar phenomenon was observed in all cases studied here. However, the results on T_g were slightly surprising, because the limit of encapsulation for dendrimers **G3**, **G3,5** and **G4** was 3 molecules of **1** per macromolecule, i.e. lower in comparison with **G2,5**, for which 6 molecules of **1** per molecule of **G2,5**, showed increased value of T_g. However it confirms the ^{1}H NMR results on solubilization in methanol, which also showed that **G2,5** interacts with more molecules of guest than larger hosts. This might be due to different types of interaction between host and **1**. Vitamin C, unlike hydrophobic *8-methoxypsoralene* is able to form ion-pair complex, this interaction prevailing in case of full-generation dendrimers **G3** and **G4**. The ion-pairs decompose when solvent is removed from solution upon preparation of neat samples. Besides, the larger dendrimers are used, the more dense are the cavities of host and self organization of vitamin C crystals prevails over weak intramolecular interaction within host-guest complex. Furthermore we noticed that homogeneous samples of host-guest complexes formed between PAMAM dendrimers and **1** can be obtained in every case when the stoichiometry is maintained as 3:1. These complexes were then used to perform transdermal permeability experiments on skin-model membranes (see below).

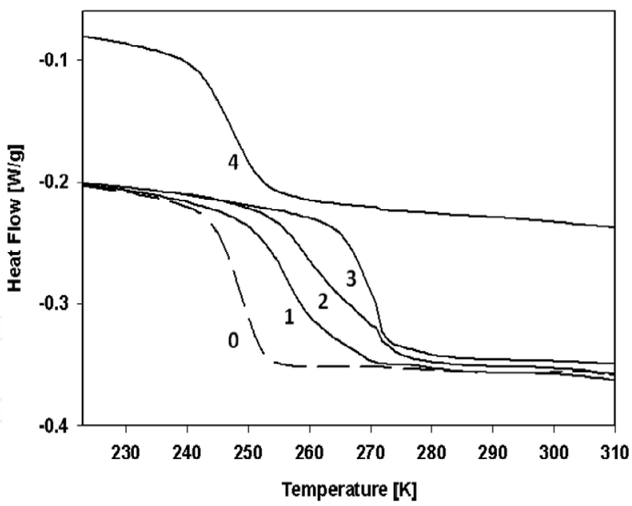

Fig. 4. The DSC curves for **1** : **G4** mixtures at molar ratio (from bottom to top): 0:1; 1:1; 2:1; 3:1; 4:1.

3.2.3 Permeation studies

The permeation experiments of **1** through PVDF and PES membranes using **1** dispersed in o/w emulsion indicated a typical change in the flux of **1**; after 0.5 hr induction time, the flux rapidly grew finally stabilizing within 1.5 hour of experiment.

Addition of PAMAM dendrimers of **G2**, **G2,5**, **G3**, **G3,5**, and **G4** only slightly influenced the rate of diffusion through PVDS membrane. The experiments were conducted until ca 50% of **1** was transferred through PVDS and the time of 10% transfer, $\tau_{0.1}$. was used as reference in all the experiments. Addition of smaller size dendrimers **G2**, **G2,5**, **G3** caused elongation of $\tau_{0.1}$, presumably due to host-guest complexes diffusion or diffusion of **1** preliminarily released from them in emulsion. The larger size dendrimers **G3,5**, and **G4** had no such impact (Figure 5a). Generally the diffusion of **1** through PVDF was fast, with 10% of **1** transferred within ca 1 hour. Unlikely, the diffusion through much thicker PES membrane occurred slower being strongly affected by the added dendrimer (Figure 5b). This influence could be clearly demonstrated by comparison of $\tau_{0.1}$ (Figure 6 and Table 2). The slowest diffusion was found in case of **1**-**G2,5** composition. The diffusion rate was diminished by a factor of 4 if compared with **1** itself. **G3** considerably decreased the rate by a factor of 3.7, while other dendrimers, larger **G3,5** and **G4** and smaller **G2**, showed lower impact. The results obtained for diffusion experiments through PES confirm the PVDF ones, nevetheless remarkable differentiation of diffusion efficiency was found in case of PES.

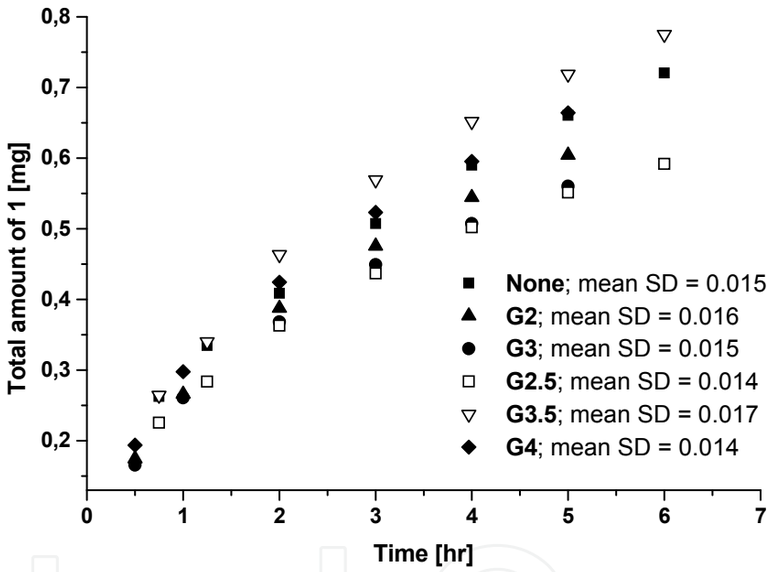

Fig. 5a. The cumulated amount of **1** diffused through PVDF membrane vs time. The load of **1** was 2.8 (± 0.2) mg in every case.

Comparing the supposed stoichiometry of host-guest complexes based on DSC measurements as well as remarkable solubilization of **1** in methanol containing **G2,5** and **G3,5** we can assume that these dendrimers form the most stable host-guest complexes, with **1** encapsulated within macromolecule, while **G3** and **G4** encapsulate **1** weakly to give the 3:1 **1**: **Gn** complexes. Moreover, **1** interacts with **G2**, **G3** and **G4** surface amine groups with this interaction prevailing on encapsulation. When **1** is released from ionic complexes with **Gn**, it diffuses similarly like from emulsion. When the whole host-guest complex diffuse, the rate is considerably lower. This phenomenon is quite opposite to what was observed for the systems in which PAMAM dendrimers were used as solubilizers of water-insoluble *psoralene* or *riboflavin* (Borowska et al, 2010, Filipowicz and Wołowiec, 2011); in these cases the transdermal diffusion of these compounds was enhanced. Both of these behaviors are

useful for controlling transdermal diffusion of dermatologically important agents. Finally the emulsion containing vitamin and dendrimeic carrier can be easily prepared by addition of pre-formed host-guest complex in ethanol and addition of oily complex to the prepared emulsion. Such prepared creams may carry both water-soluble and water-insoluble vitamins, as well as some vitamin derivatives bonded covalently, like retinal, pyridoxal or biotin. These bioconjugates are currently under investigation in our laboratory.

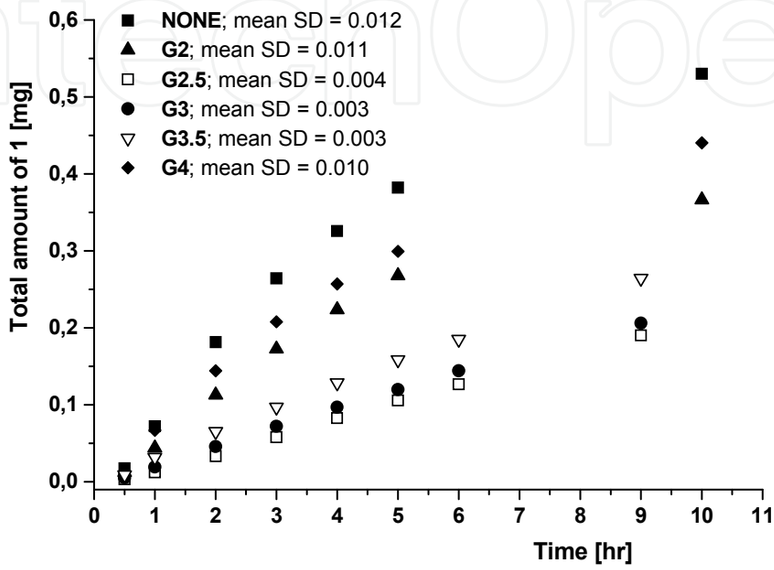

Fig. 5b. The cumulated amount of **1** diffused through PES membrane vs time. The load of **1** was 2.8 (± 0.2) mg in every case.

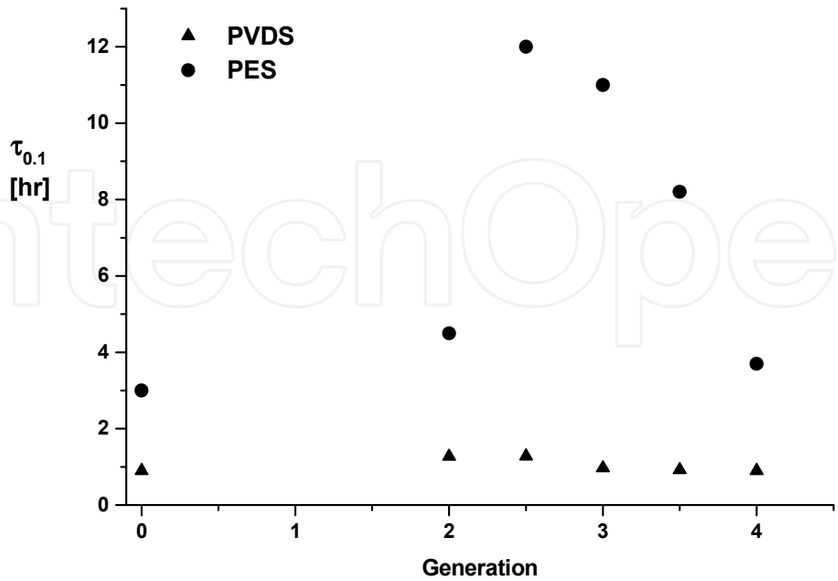

Fig. 6. The dependence of $\tau_{0.1}$ in experiments of transdermal diffusion through PVDS and PES membranes in function of PAMAM dendrimer generation.

Sample (o/w)	$\tau_{0.1}$ [hr]	
	PVDS	PES
1	0.9 (±0.12)	3.0 (±0.21)
1-G2	1.3 (±0.15)	4.5 (±0.26)
1-G2.5	1.3 (±0.15)	12.0 (±0.29)
1-G3	1.0 (±0.13)	11.0 (±0.28)
1-G3.5	0.9 (±0.12)	8.2 (±0.20)
1-G4	0.9 (±0.12)	3.7 (±0.22)

Table 2. The time of 10% transfer of **1** ($\tau_{0.1}$) from emulsions containing dendrimers.

4. Conclusions

1. PAMAM dendrimers influence the solubility of ascorbic acid (**1**) in methanol. The solubility of ionic complexes between **1** and full-generation PAMAM dendrimers in methanol is lower in comparison with **1** itself. 3:1 stoichiometry adducts between vitamin **1** : **G3** and **1** : **G4**, can be isolated from methanolic solutions as homogeneous, solvent-free oily complexes.
2. Half-generation PAMAM dendrimers: **G2,5** and **G3,5** solubilize **1** in methanol due to non-specific interaction up to *ca* 150 molecules of **1** per molecule of dendrimer.
3. The ionic interactions, encapsultation, and surface absorption of **1** by PAMAM dendrimers result in host-guest complexes formation. Based upon DSC measurements the stoiochiometry of the homogeneous, oily complexes was determined as 6:1 (for **1** : **G2,5**) and 3:1 (for **1** : **G3**, **1** : **G3,5** and **1** : **G4**).
4. When 10% of **1** per dendrimer compositions are placed in o/w emulsions, the permeation profile of **1** through the polyvinyldifluoride (PVDF) and pig ear skin (PES) membranes depends on the presence and size of dendrimer used as vitamin carrier in the emulsion. Transdermal diffusion through PES is considerably slowed down by dendrimers according to the order:

$$G2,5 \cong G3 > G3,5 >> G2 \cong G4 > \text{none}$$

5. Finally, the **G2,5** and **G3** dendrimers can be applied as transdermal carriers to control vitamin C release kinetics from emulsions or hydrogels. Vitamin C retardation is surprisingly in contrast with previously studied water insoluble 8-methoxypsoralene or riboflavin which showed a promotion of the diffusion process.

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Stoichiometry and Research - The Importance of Quantity in Biomedicine

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The aim of this book is to provide an overview of the importance of stoichiometry in the biomedical field. It proposes a collection of selected research articles and reviews which provide up-to-date information related to stoichiometry at various levels. The first section deals with host-guest chemistry, focusing on selected calixarenes, cyclodextrins and crown ethers derivatives. In the second and third sections the book presents some issues concerning stoichiometry of metal complexes and lipids and polymers architecture. The fourth section aims to clarify the role of stoichiometry in the determination of protein interactions, while in the fifth section some selected experimental techniques applied to specific systems are introduced. The last section of the book is an attempt at showing some interesting connections between biomedicine and the environment, introducing the concept of biological stoichiometry. On this basis, the present volume would definitely be an ideal source of scientific information to researchers and scientists involved in biomedicine, biochemistry and other areas involving stoichiometry evaluation.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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